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Avgift
Fee

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Methods for determining brain antigens

5

The present invention relates methods for diagnosis and follow-up of patients with cerebral dysfunction, by determining the presence of the brain protein S-100. The invention also relates to peptides comprising useful antigenic determinants from S-100 as well as monoclonal antibodies binding to these peptides.

10

As is known, the nervous system contains a number of proteins unique to its various cellular elements. The cellular disruption of nervous tissue and cells of neural origin, by any pathogenic process, trauma or by neurological diseases, results in the release of normal soluble endogenous cytoplasmic proteins into the cerebral extracellular fluid and ultimately to other

15 body fluids including the cerebrospinal fluid (CSF) and blood (serum and plasma). Examples of representative soluble small molecule weight proteins of this type can be found in the S100 protein family. A review of this family can be found in Zimmer et al., Brain Research Bulletin, Vol. 37, pp 417-429, 1995.

20 Following disruption of cell membranes, these proteins are released into the extracellular fluid in accordance with a time course and in quantities relative to the pathogenesis of the disease process or the extent of the brain tissue damages. The proteins diffuse into the CSF and then the blood or directly into the blood. The above mentioned cell membrane disruption is reflected by the blood plasmaor serum levels of one or more of these antigens and mar-

25 kers. These protein antigens have the advantage of being stable and specific, not only for the brain, but for the cellular components in the brain. By following the relative release of the various nervous system protein antigens, it is possible to deduce the kind of destructive process occuring in the course of neurological diseases and/or the extent of possible brain tissue damages. Information of this type permits the diagnosis, evaluation of severity and rate of

30 progression of the above mentioned dieases and damages.

It is previously known to determine the amount of S-100 proteins in a clinical sample. US-A-4 654 313 discloses a radioimmunological assay method for S-100 protein. The patent document does neither mention anything about different kinds of S100-proteins nor about on

35 which epitopes the assay method is based The detection limit is declared to be 0 20 ng/ml

but concentrations between 1.5 and 2.5 ng/ml is required in order to have less than 10% false positives. This concentration is rather high. Moreover, in some countries it is not permitted to use radioactive methods in clinical assays.

5   It is also known to determine S-100 proteins by using ELISA-related methods. GB-A-2 109 931 discloses a solid-phase immunoanalysis method comprising the use of enzyme-labelled antigens and particles coated with protein A on which antibodies are bound. S-100 proteins are only mentioned in claim 8 and nothing is revealed about the sensitivity of the method. JP-A-6/109 734 describes a method suitable for analysing S-100, using a first kind of antibody

10   fixed to magnetic particles, and a second kind of labelled antibody. The detection limit is stated to be 0.02 ng/ml. It is not apparent from the English abstract which one of the proteins in the S-100 family that can be analysed, and nothing is disclosed about any suitable epitopes.

15   The complexity of clinical samples is often a serious problem. An assay method may give excellent results with artificial samples in the laboratory but quite a number of unreliable results might be obtained when the method is tested under clinical conditions. When it comes to immunological assays the problems are often caused by an improper selection of antigenic determinants. An antibody in an assay, comprising the use of two different antibodies, may

20   be a hindrance to the other antibody when bound to the antigen to be determined. An improper selection of epitope for an antibody involved in the detection process may result in that the detection goup is completely or partially embedded in a protein complex and not available for detection. Different proteins present in the sample might interfere.

25   There is always a need for improvment of methods for analysing substances of medical intrest in clinical samples. An ideal clinical assay method should be quick, accurate and possible to perform with all types of clinical samples without degeneration of the accuracy for certain types of specimen. This applies to determination of S-100 proteins as well as other substances of medical interest.

30

Summary of the invention

Now it has turned out that by using antibodies directed to epitopes in the region from ser1 to asn38 and from thr82 to glu93 of the amino acid sequence of human S-100β, an improved

35   clinical assay method for determining S-100 proteins is obtained. Hence, the main object of

the present invention is an assay method using monoclonal antibodies directed to these epitopes. Another object of the present invention relates to short peptides having sequences corresponding t parts of the amino acid sequence of the human S-100β protein from ser1 to asn38 and from thr82 to glu93. Yet another object of the present invention relates to

5 analytical kits for carrying out the assay methods.

Detailed description of the invention

As already mentioned above it is often very difficult to outline methods for analysing clinical

10 samples. It is neccesary that the method has a high sensitivity and gives accurate results. It is also very important that known and unknown constituents of the sample other than the analyte do not influence the results. The present invention relates to an immunological assay method for determining the presence and/or content of human S-100 protein based upon a selection of suitable S-100 epitopes and corresponding antibodies which fulfil the above

15 mentioned requirements.

It has turned out that the selected epitope combinations provides tests and test kits where:

1.   a high sensitivity is achieved;

2.   the antibodies of the kit binds equally strong to the internal standard as to the

20   analyte in the clinical sample;

3.   the epitopes are chosen in such a way that the different antibodies do not
          interfere with each other when they bind to the analyte, i.e. that the epitopes
          are situated sufficiently distant from each other.

25 The epitopes of the present invention are all comprised in the human S-100β protein. Epitopes present within the amino acid sequences:
SELEKAMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 2)
and
TACHEFFEHE (SEQ.ID.NO. 3)

30 are preferred. Particularly preferred are epitopes comprised within the peptide
AMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 4) and especially
within the peptides REGDKHKLKKSELKEL (SEQ. ID. NO. 5) and EFFEHE (SEQ ID.
NO. 6).

The disclosed epitopes are, among all, used to construct peptides for inducing the formation of suitable antibodies on which the claimed assay method is based. These peptides mostly consist of up to 40 amino acids. The whole amino acid sequence of a peptide according to the present invention is derived from human S-100β. These peptides may comprise variants

5   wherein the original amino acid sequence is modified or altered by insertion, addition, substitution, inversion or deletion which preferably show at least 90% homology with the sequence of SEQ. ID. NO. 1 and retain essentially the same immunological properties. The peptides may also comprise multiples of certain epitopes, and in this case their sequence length may exceed 40 amino acids.

10

By the expression "sub-fragment" is meant a peptide sequence having a length of at least 6 amino acids.

The epitopes can also be used to construct fusion peptides comprising at least two distinct

15   epitopes which, among all, can be used as internal standard in immunoassays.

Abbreviations.

The following abbreviations are used:

20	S100	-S100β
	RT	-Room Temperature
	BSA	-Bovine Serum Albumin
	Mab(s)	-Monoclonal antibody(ies)
	kD	-kiloDalton
25	ECL	-Enhanced Chemiluminescent Assay
	CBB	-Commassie Brilliant Blue
	LIA	-Luminometric Immuno Assay
	IRMA	-Immuno Radio Metric Assay
	ELISA	-Enzyme Linked ImmunoSorbent Assay
30	SDS-PAGE	-SodiumDodecylSulfate - PolyAcrylamideGelElectrophoresis
	PBS	-Phosphate Buffered Saline
	RLU	-Relative Light Units
	NHS	-N-HydroxySuccinimide
	EDC	-N-ethyl-N'-(dimethylaminopropyl)-carbodiimide
35	RAMFc	-RabbitAnti-MouseFc antibody

EDTA	-EtylenDiamineTetraAcetic acid
NaCl	-Sodium Chloride
NaN₃	-Sodium azide
iv.	-intravenously
5	aa	-amino acid
ng	-nanogram
ml	-millilitre
mg	-milligram
HRP	-HorseRadish Peroxidase
10	h	-hour(s)
min	-minute(s)
sec	-second(s)

15 **Experimental details common to all test procedures**

The peptides were prepared by the methods disclosed in Merrifield (1963), J. Am. Chem. Soc., vol. 85, p 2149; Gutte et al.(1971), J. Biol Chem vol. 246, p. 1922; and Carpino et al. (1970), J Am Chem Soc vol. 92, p. 5748.

20

The monoclonal antibodies were prepared by the method-according to Köhler et al.(1975), Nature vol. 256, p. 495; and Harlow et al (1988), Antibodies. A Laboratory Manual, Cold Spring Harbor, p. 139.

25 <u>Antigen and Standard preparations</u>

Procedure for preparation and purification of S100 antigen prior to immunisation of Balb/c mice was according to Moore (Biochim. Biophys. Res. Comm. 1965, 19: 739 - 744) with a slight modification according to Haglid & Stavrou (J. Neurochem.1973, 20:1523-1532).
30 Briefly, bovine brain was homogenised in Tris buffer, pH 7.2. The homogenate was centrifuged at 10.000 r.p.m. and the clear supernatant was used for further purification by ammonium sulphate precipitation The fraction still soluble after saturation by ammonium sulphate was dialysed and purified by separation on a Sephadex G150 Sepharose (Pharmacia Biotech AB, Uppsala Sweden)chromatographic column followed by separation on a DEAE-sepha-

dex (ionic exchange) column (Pharmacia Biotech AB, Uppsala Sweden). The fraction eluted by 0.3 - 0.4 M NaCl was collected, desalted, lyophilised and used for further experiments.

Hybridoma construction.

5

Balb/c mice were immunised with purified S100ββ intraperitonially in Freund's complete adjuvant and were given booster iv. injection 6 weeks later during 3 consecutive days. The spleen was removed on the fourth day after last injection and prepared for fusion. The myeloma cell line Sp2/0-Ag14 was used for fusion of Balb/c spleen cells.

10

Antibody purification and subclass determination.

Monoclonal antibodies were identified, extracted and purified from hybridoma supernatant according to Harlow & Lane Eds. in ANTIBODIES A LABORATORY MANUAL, Cold Spring

15 Harbour Laboratory Press, New York 298-299 & 311.

Briefly, positive hybridoma clones carrying supernatant with specific antibodies were identified using ELISA with microtitreplate wells coated with S100ββ.

Immunoglobulins were precipitated using saturated ammoniumsulphate and dialysed against 1.5 M Glycine, 3 M NaCl, pH 8.9. Dialysed material were affinity chromatography purified

20 on an protein-A Sepharose (Pharmacia Biotech AB, Uppsala Sweden) column. Fractions were neutralised by addition of small volumes of 1M Tris pH 8.0.

Epitope mapping.

25

S100β (monomer) epitopes for respective antibody was investigated by use of a synthetic peptide library. Peptides were linked to nitro-cellulose filter membrane via an amide link, according to the manufacturer (Research Genetics', USA) and covers all ninety-one aa in the protein. In total the library consisted of thirty-one, all except one being ten aa-residues long

30 synthetic peptides. Each peptide was consecutively shifted three aa towards the -COOH terminal end of the protein Positive antibody-binding was indicated by the use of a second anti-mouse antibody conjugated with HRP and detected using an ECL assay (Amersham, UK).

Results:

35 Two binding sequences were found

Epitope 1
AMVALIDVFHQYSGREGDKHKLKKSELKELINN (residues 6-38)(SEQ. ID. NO.4)

5   and

Epitope 2
EFFEHE (residues No 86-91) (SEQ. ID. NO. 6)

10

Antibody reactivity.

Purified antibodies reacting with the epitopes were checked for reactivity and affinity using
the BIAcore™ system (Pharmacia Biosensor AB, Uppsala Sweden).

15   Briefly, in order to test the specificity of the antibodies, the RAMFc was immobilised onto
the sensor chip CM5 NHS-ester activated surface, according to standard procedure, to pro-
vide approximately 600 RLU. Then each Mab was bound to the RAMFc surface to approxi-
mately 300RLU, followed by the S100αα and the S100 standard (consisting of 50%
S100αβ and 50% S100ββ) in separate experiments. All reactions were carried out in con-

20   tinuos flow of the phosphate buffer . The kinetics between antibodies and antigen was done
similarly . S100 antigen was added to the chips at 200-450nM for reactivity measurements
of the antibody intended for the solid phase and at 1000-1500nM for measurements of the
antibody intended for tracers Kinetics was determined using the BIAcore™ Kinetic evalua-
tion 2.1, software (Pharmacia Biosensor AB, Uppsala Sweden). It can be concluded from

25   the reactivity profile that the antibodies reactive with the epitopes are specific for the β-
containing forms of S100 and not the α-containing form.

Example 1
Development of an immunoluminometric procedure

30

Tracer antibody was conjugated with luminol according to Vojtesek et al. 1992, J Immunol.
Methods 151: 237-244). Briefly, ABEI (Sigma, St Louis, Ms) was linked with a diactivated
ester (Etylenglykolbis-succinidyl succinat, EGS) The ABEI-EGS-conjugate was next mixed
with monoclonal antiS100-antibody in an approximately 50 5 molar ratio in 100µl of PBS

pH 7.4, containing 15% acetonitrile and incubated 1 h at room temperature. The ABEI-conjugated antibody was purified on a Sephacryl®S 300 HR (Pharmacia Biotech AB, Uppsala Sweden) gelfiltration column, and appropriate fractions were pooled and diluted in phosphate-buffer.

5

Preparation of antibody coated tubes for LIA.

Polystyrene tubes (Greiner, Germany) were incubated overnight at room temperature with 3μg of S100-antibodies in 300 μl of PBS pH 7.5. The tubes were washed with 0.1%

10   Tween20® in PBS. Next, tubes were blocked with a solution containing 0.9%BSA and 4% Saccarose and incubated for 24h. The solution was aspirated and the tubes allowed to dry.

LIA test procedure.

15   The test was conducted in a two step procedure by incubating 100μl of patient body fluid in antibody coated tubes, or S100 standard with 100 μl of diluent (PBS + 5%BSA) and incubated at room temperature. After washing 200 μl of the luminol-labelled antibody was added and a further 2 h of incubation was performed before measurement. After another washing the luminescence was developed using the LIA-mat starter service kit (Byk-Sangtec,

20   Diezenbach Germany) and immediately measured as integrals over a period of 5 sec in luminometer (Berthold, Germany) In order to convert the obtained light signal into concentration of S100 measurements on patient samples were compared with measurements on solutions with known concentrations of S100 (standards) The detection limit (zero standard +3 standard deviations) was approximately 0.01 μg/l

25

Example 2

Development of an ELISA test procedure.

As tracer antibody was used monoclonal antiS100 antibody conjugated with β-galactosidase

5   according to Harlow & Lane Eds. in ANTIBODIES A LABORATORY MANUAL, Cold Spring
Harbour Laboratory Press, New York page 351.

Preparation of antibody coated microtiter wells for ELISA.

10

Microtiterplatewells (Corning, Denmark) were incubated overnight at +4°C with 2.5μg of
microtiter wells were finally washed three times with 0.05% Tween20® and air dried before
use.

15   ELISA test procedure.

The ELISA was conducted in a multiple step incubation procedure.

100 μl of 1:1 diluted patient sample or 100μl of S100 standard (0 - 20 μg/ml) was added to
the wells.

20   The plate was incubated for 1.5h at RT under shaking.

The plates were washed three times with 300μl 0.05% Tween20® in PBS.

100 μl of alkaline phosphatase conjugated tracer antibody was added and a further 1.5h of
incubation on a shaker was performed.

The wells were then washed three times with 0.05% Tween20® in PBS.

25   100μl of a 5% o-nitro-phenyl-β-galactoside substrate solution was added and the plates
were incubated with substrate for another forty-five minutes and colour is developed.

The colour development was stopped by the addition of 100μl 0.66M Na_2CO_3.

Each well of the plate was read at 405nm in a standard microtiterplate reader. In order to
convert the obtained colour signal into concentration of S100, measurements on patient

30   samples were compared with measurements on solutions with known concentrations of
S100 (standards) The detection limit (zero standard +3 standard deviations) was approxi-
mately 0.2 μg/l

Result:

Standard (µg/l)	0	0.5	1.5	5	15
A 405	0.088	0.147	0.244	0.675	1.196

5

Example 3

Development of an immunoradiometric (IRMA) test procedure.

IRMA tracer antibody conjugation.

10   A monoclonal antiS100 antibody was conjugated with Iodine using the Chloramine T method according to Greenwood et al. (Biochem. J. 1963, 89:114-123). The specific activity was determined to be 520±80 MBq/mg

Preparation of antibody coated to polystyrene beads

Monoclonal anti S100 antibodies were coupled to polystyrene beads by the Glutaraldehyde coupling method according to Harlow & Lane Eds. in ANTIBODIES, A LABORATORY
5   MANUAL, Cold Spring Harbour Laboratory Press, New York, 533 & 536-537. Final blocking was by 1% BSA, 0.1% NaN$_3$ in PBS pH7.5.

IRMA test procedure.

10   100µl of patient sample or standard was added to polystyrene tubes together with 100µl PBS diluent. One polystyrene coated bead was added to each tube followed by incubation for 1 h at RT on a shaker. Next the beads were washed once with 2ml of demineralised water and 200µl of I-125 labelled tracer antibody was added and the tubes were incubated a further 2h on a shaker. After washing the radioactive signal on the bead was measured in a
15   standard γ-counter. In order to convert the obtained radioactive signal into concentration of S100, measurements on patient samples were compared with measurements on solutions with known concentrations of S100 (standards). The detection limit (zero standard +3 standard deviations) was approximately 0.1 µg/l.

20

Example 4
Use of IRMA test procedure for assay of S100 in serum from melanoma patients.

25   The S100 based test procedure was applied on clinical questions relating to melanoma Blood samples from patients with melanoma of various stages of cancer progression were collected in serum collecting tubes Samples were then frozen and treated according to the test procedure described above in Example 3
Results:.
30   Relationship to staging
Clinical Stage I vs Clinical Stage II. In a study of 577 patients the geometric mean for Stage I was found to be 0 12 µg/l and for Stage II the geometric mean was found to be 0 33 µg/l p-value < 0.001

Example 5

Use of IRMA test procedure for assay of S100 in serum from melanoma patients.

5   The S100 based test procedure was applied on clinical questions relating to melanoma. Blood samples from patients with melanoma of various stages of cancer progression were collected in serum collecting tubes. Samples were then frozen and treated according to the test procedure described above in Example 3.

Results:

Relationship to survival

10 Clinical Stage I vs Clinical Stage II and III. In a study with respect to survival performed on 643 patients the relative hazard and 95% confidence interval was calculated. The relative hazard was found to be 12.3 and the confidence interval 5.6-27.2 with a p-value of <0.001

Example 6

Use of the S100 LIA-method for evaluation of the influence of extra corporal circulation equipment on the brain.

The S100 based test procedure in Example 1 was applied on monitoring cerebral injury

5 following extra corporeal circulation (ECC). Blood samples from patients undergoing extra corporeal circulation were collected in serum tubes and treated according " Test procedure".

Results

	Before start of ECC	End of ECC	1 day after surgery	2 days after surgery
S100 levels µg/l	0	1,67	0,21	0,13

10 In this group of patients the level of S100 in serum was elevated for at least 2 days after surgery.

Uncomplicated cases should return to normal levels within the first 24 hours (Ref P. Johnson et al. J. Cardiothor .Vasc Anaesthesia, 9:6 (1995) 694-99).

15

Example 7.

Use of LIA test procedure for assay of S100 in serum from melanoma patients.

The S100 based test procedure was applied on clinical questions relating to melanoma. Blood samples from patients with melanoma of various stages of cancer progression and

20 blood donors were collected in serum collecting tubes. Samples were frozen and treated according to the test procedure described above in Example 1.

Result: Of 136 patients with various stages of melanoma 25 had a level of S100 below 0.08 and of 100 blood donors tested on the same occasion 7 had a level equal to or above 0.08

25 µg/l.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

  (i) APPLICANT:
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  (ii) TITLE OF INVENTION: Methods for determining brain antigens

  (iii) NUMBER OF SEQUENCES: 8

  (iv) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Floppy disk
    (B) COMPUTER: IBM PC compatible
    (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 91 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS:
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

  (iii) HYPOTHETICAL: NO

  (iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

  (vi) ORIGINAL SOURCE:
    (A) ORGANISM: Homo sapiens

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Glu Leu Glu Lys Ala Val Val Ala Leu Ile Asp Val Phe His Gln
1   5   10   15

Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu
   20   25   30

Lys Glu Leu Ile Asn Asn Glu Leu Ser His Phe Leu Glu Glu Ile Lys
    35   40   45

Glu Gln Glu Val Val Asp Lys Val Asn Glu Thr Leu Asp Ser Asp Gly
    50   55   60

Asp Gly Glu Cys Asp Phe Gln Glu Phe Met Ala Phe Val Ala Met Ile
65   70   75   80

Thr Thr Ala Cys His Glu Phe Phe Glu His Glu
    85   90

(2) INFORMATION FOR SEQ ID NO: 2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: N-terminal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

    Ser Glu Leu Glu Lys Ala Met Val Ala Leu Ile Asp Val Phe His Gln
    1   5   10   15

    Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu
        20   25   30

    Lys Glu Leu Ile Asn Asn
        35

(2) INFORMATION FOR SEQ ID NO: 3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Thr Ala Cys His Glu Phe Phe Glu His Glu
1   5   10

(2) INFORMATION FOR SEQ ID NO: 4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: internal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Met Val Ala Leu Ile Asp Val Phe His Gln Tyr Ser Gly Arg Glu
1   5   10   15

Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu Ile Asn
        20   25   30

Asn

(2) INFORMATION FOR SEQ ID NO: 5:

    (i) SEQUENCE CHARACTERISTICS

      (A) LENGTH: 16 amino acids
      (B) TYPE: amin acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu
1   5   10   15

(2) INFORMATION FOR SEQ ID NO: 6:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 6 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo sapiens

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Glu Phe Phe Glu His Glu
1   5

(2) INFORMATION FOR SEQ ID NO: 7:

   (i) SEQUENCE CHARACTERISTICS

        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: internal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

    Asp Lys His Lys Leu Lys Lys Ser Glu Leu
    1   5   10

(2) INFORMATION FOR SEQ ID NO: 8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: internal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

    Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu
    1   5   10

Claims

1. A peptide consisting of at least one sub-fragment of the human S-100β protein having from 6 to 40 amino acids, where said sub-fragments show at least 90% homology with the sequence according to SEQ. ID. NO. 1 and retain essentially the same immunological properties.

2. A peptide according to claim 1, characterized in that the sub-fragments are derived from the amino acid sequence:

SELEKAMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 2)

and/or the amino acid sequence

TACHEFFEHE (SEQ. ID. NO. 3).

3. A peptide according to claim 2 characterized in that the sub-fragments are derived from the amino acid sequence:

SELEKAMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 2).

4. A peptide according to claim 3, which is

REGDKHKLKK (SEQ. ID. NO. 5);
DKHKLKKSEL (SEQ. ID. NO. 7), or
KLKKSELKEL (SEQ. ID. NO. 8).

5. A peptide according to claim 2, characterized in that the sub-fragments are derived from the amino acid sequence.

TACHEFFEHE (SEQ. ID. NO. 3).

6. A peptide according to claim 5, which is

EFFEHE (SEQ. ID. NO. 6)

7. A peptide according to anyone of claims 1 or 2 consisting of multiples of sub-fragments derived from the sequence of SEQ. ID. NO. 1.

5 8. A peptide according to claim 2, characterized in that it consists of at least one sub-fragment derived from the sequence according to SEQ. ID. NO. 2 and at least one sub-fragment derived from the sequence according to SEQ. ID. NO. 3.

9. A monoclonal antibody or a fragment of such an antibody specifically binding a peptide
10 according to anyone of the preceeding claims.

10. A monoclonal antibody or an antibody fragment according to claim 9, specifically binding a peptide according to claim 3.

15 11. A monoclonal antibody or an antibody fragment according to claim 9, specifically binding a peptide according to claim 5.

12. Use of a monoclonal antibody or an antibody fragment according to anyone of claims 9 - 11 in immunological assay methods.
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13. Use of a peptide according to anyone of claims 1-8 for eliciting antibodies.

14. Use of a peptide according to anyone of claims 1 - 8 in immunological assay methods.

25 15. A method of determining the presence of human S-100 protein in a sample comprising the steps of:

letting the sample to be analyzed immunologically react with a first monoclonal antibody according to claim 3, said first antibody being coupled to a carrier;

letting the sample immunologically react with a second monoclonal antibody
30 according to claim 5, said second monoclonal antibody being provided with detection means,

Washing, and

detecting the amount of S-100 protein in the sample.

16. A method according to claim 15 where the detection means is a group having the ability of emitting luminescence.

17. A method according to claim 15, where the carrier is a magnetic particle.

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18. A kit for determining the presence of human S-100 protein in a sample, comprising a peptide according to anyone of claims 1 - 8 and/or an antibody according to anyone of claims 9 - 11.

Abstract

An assay method for determining the presence of of the brain protein S-100 in a clinical sample which uses antibodies directed to epitopes in the region from ser1 to asn38 and from thr82 to glu93 of the amino acid sequence of the β subunit of human S100B is provided.

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